RESOURCE UTILIZATION IN POSTLARVAL BROWN SHRIMP: THE POTENTIAL IMPORTANCE OF HERBIVORY

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ABSTRACT

Estuaries provide a nursery ground for developing penaeid shrimp, but the food sources utilized and the patterns of feeding employed by these animals while occcupying this habitat are not known. Laboratory feeding experiments were conducted to investigate the herbivory potential of postlarval brown shrimp (Penaeus aztecus Ives) inhabiting a Spartina alterniflora Loisel salt marsh. Plant materials fed to shrimp included Skeletonema costatum (Greville) Cleve, Isochrysis sp., Spartina detritus, and Spartina epiphytes. Growth and survivorship were assessed in 16 treatments derived from all possible combinations of the four materials. After 16 days the greatest increases in length and weight occurred in those treatments containing Skeletonema costatum (Skeletonema Group) followed by Spartina epiphytes exclusive of the presence of Skeletonema (Epiphyte Group). Shrimp growth did not occur in beakers with (1) no food, (2) Isochrysis sp. or Spartina detritus alone or (3) Isochrysis and detritus in combination (Isochrysis-Detritus-No Food Group). Survivorship and growth were significantly higher in the Skeletonema and Epiphyte Groups compared to the Isochrysis-Detritus-No Food Group.

Carbon assimilation was monitored at 4-day intervals with stable carbon isotopes (delta C-13) for shrimp reared on single food sources or a combination of all foods. Assimilation corresponded well to growth in those treatments where the greatest increases in weight occurred, most rapid carbon turnover in shrimp fed Skeletonema or all

foods, and indicated that the half-life of tissue carbon was reached before the first doubling of weight. Food preferences were assessed with those materials which promoted growth (i.e. Skeletonema and epiphytes). There were no differences in observed preference patterns between day and night trials. Although there was no preference for Skeletonema and epiphytes together compared to epiphytes alone, selection for both of these materials was greater than for Skeletonema alone.

The results of this study indicated that (i) diatoms, such as Skeletonema, as well as epiphytes of Spartina, are potential sources of nutrition for postlarval P. aztecus in Spartina marshes, (ii) delta C-13 analysis is a useful tool for identifying assimilable foods, (iii) plants in the marsh may be more important for metabolic maintenance than for growth, and (iv) postlarval brown shrimp may have a primary preference for substrate which is not directly related to apparent plant food value.

CHAPTER 1

BACKGROUND

Salt marshes are generally considered to be areas of comparatively low macrofaunal diversity where monospecific stands of vascular plants often predominate (Wiegart and Pomeroy, 1981). Although primary production, in the form of marsh grasses and benthic and epiphytic algae, is usually higher than in terrestrial communities (Blum, 1968; Keefe, 1972), these plants are not thought to provide enough niche variety to support higher order trophic schemes (Wiegart and Pomeroy, 1981). Additionally, few macrofaunal species are considered to be specialized for feeding on the much smaller algal cells prevalent in the salt marsh (Wiegart and Pomeroy, 1981). Despite these possible food limitations, many species of fish and shellfish seasonally utilize salt marshes as nurseries for their developing larvae, postlarvae, and juveniles (Weinstein, 1979). To fully understand the capacity of the salt marsh to periodically harbor large numbers of transient macrofaunal species and the interactions which occur between individuals, both permanent residents and migrants, it is important to obtain a thorough knowledge of the resources consumed and patterns of feeding employed by animals occupying this habitat

Penaeus aztecus Ives (brown or grooved shrimp), is a common crustacean of the upper Texas coast which utilizes the estuarine nursery grounds, both salt marshes and seagrasses, during the postlarval and juvenile stages of its life-cycle (Pearson, 1939; Williams, 1955). Previous field studies conducted in these habitats, in an effort to identify the resources consumed and nutritionally

valuable, indicated that penaeid shrimp ingested many forms of plant material (Jones, 1973; Brisson and Pace, 1978; George, 1978; Chong and Sasekumar, 1981; Hughes and Sherr, 1983; Kitting et al., 1984). Jones (1973) suggested that young juvenile (25-50 mm) P. aztecus were omniverous consuming bacteria, algae, Spartina detritus, and live animals. Other researchers, such as Condrey et al. (1972), determined that benthic algal and detrital microbial communities on dead Spartina were the most likely food sources. In addition to these plant materials, diatoms were also common in the diet of postlarval and juvenile penaeids (Jones, 1973; George, 1978; Chong and Sasekumar, 1981). Recent studies using delta C-13 analysis indicated that epiphytic algae on seagrass blades (Kitting, et al., 1984) and phytoplankton (Hughes and Sherr, 1983) were important food sources for P. aztecus. These conclusions have been further supported by an increasing body of literature which has placed more emphasis on the role of algae as a food source in marsh and seagrass habitats (Fry and Parker, 1979; Fry, 1984).

Although the results of these past field studies implicated that plants are important dietary components for postlarval and juvenile penaeid shrimp, the percent contributions of these materials in the diet were not determined. Gut analysis was not an accurate indicator because a large percentage (up to 50%) of the food particles were often well digested and unidentifiable (Jones, 1973; George, 1978; Chong and Sasekumar, 1981). Previous laboratory studies indicated that plant

material is nutritionally valuable to penaeid shrimp (Williams, 1959; Venkataramiah et al., 1975; Moriarty, 1976), however, the relative contribution of this component to growth, assimilation, and survivorship was not investigated. In addition, laboratory studies using naturally available plant food sources, such as green algae and detritus, assumed that penaeid shrimp have no feeding preferences (Zein-Eldin, 1963; Condrey et al., 1972; Brisson and Pace, 1978). Considering that marine algae are species specific in their amino acid and sugar compositions (Fowden, 1954; Parsons et al., 1961; Hecky et al., 1973; Haug et al., 1976; Atkinson and Smith, 1983) and various concentrations of these substances differ in their ability to elicit a feeding response in penaeid prawns (Hindley, 1975), this assumption may be unjustified.

In this study, through a series of laboratory feeding experiments, I investigated aspects of herbivory in postlarval Penaeus aztecus Ives. Specifically, the ability of this species to utilize diets consisting of salt marsh plant materials was examined by addressing three questions:

1) Can postlarval P. aztecus reared exclusively on plant materials representative of those present within the salt marsh survive and grow?

2) Do postlarval P. aztecus differentially assimilate among plant food sources and do the patterns of assimilation correspond to growth?

3) What preferences, if any, do postlarval P. aztecus exhibit for plant materials and are preferences related to growth and assimilation?

In Chapter 2, I addressed the first question by monitoring growth of postlarval brown shrimp fed plant foods singly and in combinations. The plant materials fed to shrimp included Skeletonema costatum (Greville) Cleve, Isochrysis sp., Spartina detritus, and epiphytes of Spartina. The results indicated that in some instances postlarval P. aztecus were able to survive for 16 days on diets consisting solely of plant material. The most rapid growth occurred on the diatom Skeletonema costatum followed by epiphytes of Spartina. No synergistic or additive effects on growth were evident, suggesting either differential assimilation or preferential feeding, or some association between the two.

Assimilation rates and food preferences were investigated in Chapter 3. Patterns of assimilation were monitored by analyzing shrimp tissues for changes in the ratio of $^{13}C/^{12}C$, whereas preferences were assessed by providing the animals a free choice between those foods which promoted growth (i.e., Skeletonema and epiphytes).

The results indicated that assimilation corresponded well to growth in those treatments where the greatest increases in body weight occurred (i.e., Skeletonema alone and all foods in combined). Since the assimilation curves were nearly identical in Skeletonema and combination treatments, it was suggested that the shrimp were feeding preferentially. Unexpectedly, preferences did not correlate with either assimilation or growth, but instead showed selection for substrates unrelated to apparent plant food value.

CHAPTER 2

GROWTH AND SURVIVORSHIP

INTRODUCTION

The general life-cycle of the brown shrimp, Penaeus aztecus Ives, has been well documented (Pearson, 1939; Williams, 1955). This life-cycle includes offshore spawning, oceanic larval development, and migration into estuaries as postlarvae. Following rapid growth in the estuary, shrimp return to offshore areas as subadults. While estuaries presumably provide nursery conditions for developing postlarval penaeids (Williams, 1959; Gunter, 1961; Weinstein, 1979), the specific contributions of estuarine habitats are not known. One important function, however, may be the provision of abundant and necessary food resources not readily available in offshore areas.

Along the eastern seaboard and Gulf coast, salt marshes are dominated by the smooth cordgrass, Spartina alterniflora Loisel (Chapman, 1960). These marshes produce an abundance of detritus (Odum and de la Cruz, 1967), but its direct importance to the nutrition of penaeid shrimp has not been established. In some studies the nutritional value of plant detritus and its associated microorganisms has been implied by the presence of this plant-microbe complex in gut contents (Jones, 1973; George, 1978; Chong and Sasekumar, 1981). Other studies have cast doubt on the nutritional value of plant derived detritus because: 1) other plant and animal materials are often abundant in shrimp guts (Moriarty and Barclay, 1981), 2) high concentrations of P. aztecus have been found in areas where detrital production from photosynthetic sources is low (Weinstein, 1979), and 3)

marsh associated algae may be eaten and better assimilated by penaeids (Hughes and Sherr, 1983). The role of plants in the nutrition of shrimp associated with the Spartina habitat has received little attention. Field studies have indicated that penaeid shrimp not only consume plant detritus (Jones, 1973; George, 1978; Chong and Sasekumar, 1981), but also benthic algae (Jones, 1973; Brisson and Pace, 1978; Hughes and Sherr, 1983), epiphytic algae on plants (Kitting et al., 1984), and planktonic algae (Hughes and Sherr, 1983). Laboratory studies have shown that juvenile penaeids readily feed on various algae including diatoms (Williams, 1958, 1959; Zein-Eldin, 1963; Rickards, 1971; Condrey et al., 1972; Venkataramiah et al., 1975; Brisson and Pace, 1978), and digest and assimilate plant cell walls (Moriarty, 1976). In addition, maximum growth rates have usually been attained when combinations of plant and either live (Williams, 1958) or dead (Venkataramiah et al., 1975) animal material have been fed to shrimp. The presence of vegetable matter in the diet of shrimp has been suggested to be essential for high survivorship and efficient energy conversion of protein sources (Venkataramiah et al., 1975).

Based on recent studies demonstrating a strong positive correlation between juvenile shrimp densities and the presence of S. alterniflora habitat (Zimmerman et al., 1984), I hypothesized that algae and plant detritus associated with Spartina might be important in stimulating growth in developing shrimp. Although this study investigated only the potential for plant materials to promote growth

and survivorship of P. aztecus, I recognize that postlarval penaeid shrimp are probably omnivorous. Research on field populations has indicated a diet consisting of plant and animal sources, but due to the inefficiencies of gut analysis has not determined the relative importance of each of these components in terms of growth and survivorship (Jones, 1973). By taking brown shrimp postlarvae from the field and rearing these animals on representative salt marsh plant materials, this study represented a first step in determining the contribution of these food sources to the nutrition of P. aztecus.

METHODS AND MATERIALS

All P. aztecus postlarvae were captured using a hand-towed beam trawl (Renfro, 1963) either on the front beach or at one of two passes on either end of Galveston Island, Texas. Brown shrimp postlarvae migrating from open coastal waters through the passes averaged 10-12 mm (rostrum-telson) in length. Postlarvae were taken to the laboratory, identified according to Ringo and Zamora (1968) and starved for not less than 12 h nor more than 24 h before initiation of the experiments.

Four sources of plant material, representative of benthic, epiphytic, and planktonic species present within the salt marsh, were used in the experiments: Spartina detritus, epiphytes of Spartina, Skeletonema costatum, and Isochrysis sp. The epiphytic and detrital materials were collected from a Spartina marsh located in Galveston Island State Park on the West Bay side of Galveston Island. A complex,

termed Spartina detritus, of decaying vascular plant fragments and Aphanothece stagnina (Spreng.) A. Br. (a gelatinous colonial blue-green alga) was collected by straining the top 5 cm of sediment from within Spartina stands through a 250 μm sieve. Material carefully scraped with a scalpel from Spartina stems was referred to as epiphytes. These epiphytes consisted of many species of green algae, blue-green algae, and colonial diatoms. Common species of each present were Ulothrix flacca (Dillw.) Thur. and U. subflaccida Wille, Oscillatoria curviceps Ag. ex Gomont, and Nitzschia clausterium (Ehr.) Wm. Smith, respectively. After removal of visible macrofauna, a meiofaunal component (predominately nematodes) remained in treatments with detritus and epiphytes (Table 1). Preliminary experiments indicated that postlarval P. aztecus, residing for 4 days in beakers containing detritus, feed on these meiofauna (Detritus, mean=27.5 worms/sample, n=10; Detritus + Shrimp, mean=9.8 worms/sample, n=10; $F=21.59$, $d.f.=1,18$, $P<0.01$). To reduce the confounding effects of animal contaminants (Table 1), detritus and epiphyte treatments were subjected to a two-step procedure which included (1) thoroughly washing the samples with filtered sea water and (2) allowing bacteria to decompose any remaining nematodes by keeping the samples undisturbed for a minimum of 48 h. Skeletonema (a planktonic diatom) and Isochrysis (a flagellated green alga) treatments were available from algal cultures virtually free of contamination except for infrequent dinoflagellates. Thus, differences in growth and survivorship between treatments were

attributed to the ingestion of plant materials and their associated bacteria and fungi.

To prevent cannibalism, postlarvae were reared individually in 250 ml beakers filled with sea water. Initial lengths and weights were not significantly different between treatments (One-Way ANOVA; F=1.5, d.f.=15,144, P=0.34 for length; F=1.12, d.f.=15,144, P=0.11 for weight). Growth and survivorship were assessed for 16 days in 16 treatments, including all possible combinations of the four plant sources and a no food treatment. Preliminary experiments indicated that 16 days was the maximum time period that individuals could survive without addition of food. Each treatment was comprised of 10 individually contained shrimp (replicates). The foods were presented to the shrimp in concentrations exceeding that which the postlarvae could consume in a 4-day period. In treatments with Spartina epiphytes and Spartina detritus, shrimp were placed with 0.5 ml of plant material in 250 ml of filtered (5 µm) sea water. For Skeletonema and Isochrysis, initial densities in each 250 ml replicate were approximately 5×10^5 cells/ml. Plant materials were replaced and beakers thoroughly cleaned every 4 days to prevent depletion of food resources and microfloral contamination. One-half of the water in each beaker was exchanged every two days between plant replacements. Daily observations verified that all food sources were abundant during the entire 16 days. To maintain the treatments containing Skeletonema, Isochrysis, and Spartina epiphytes, 0.5 ml of 2% F/2 algae media

(Guillard, 1975) was added initially and at each plant replacement. Preliminary experiments conducted for 16 days indicated that addition of F/2 algae media had no effect on either growth or survival of the postlarvae. Comparisons between shrimp in the presence and absence of algae media found no significant differences in initial and final lengths ($F=1.75$, d.f.=1,40, $P>0.05$) and weights ($F=2.01$, d.f.=1,40, $P>0.05$), or number of days survived (Mann-Whitney U-test, $U_s=257$, $n_1=25$, $n_2=25$, $P>0.05$).

Shrimp were subjected to a 12-h light/12-h dark photoperiod, and salinity and temperature were maintained at 25 $\pm$1 ppt and 25 $\pm$1 ^{o}C to promote optimal growth (Zein-Eldin, 1963; Zein-Eldin and Aldrich, 1965; and Zein-Eldin and Griffith, 1966). To reduce evaporation and prevent shrimp from escaping the beakers, a thin polyethylene cover (plastic wrap) was placed on the surface of the water. Dissolved O_2 was monitored every other day using a YSI Model 51B oxygen meter, between 0800 and 1200 h by sampling 12 beakers (3 representatives each from Skeletonema, Isochrysis, detritus, and epiphytes). Oxygen concentration in the beakers was never less than 5 ppm and in most cases was maintained between 8-10 ppm due to photosynthesis. This was comparable to levels found in the field (range = 5.8-9.0 ppm) for samples taken at similar times of day over 16 days.

At 4-day intervals lengths and weights were determined by measuring individual live shrimp (rostrum-telson) under a dissecting microscope to the nearest 0.1 mm, blotting dry, and then weighing on a

microbalance to the nearest 0.2 mg. Earlier experiments utilizing the same protocol demonstrated that little, if any, mortality was due to the measuring and weighing procedure. Observations on mortality were taken every other day.

After 16 days, changes in lengths and weights were tested for normality (SAS Institute Inc., 1982) and homogeneity of variances (F_{max}-test; Sokal and Rohlf, 1969). Since significant departures occurred in both cases, all growth data were transformed by $\log_{10}(x+3)$ prior to analysis of variance. However, data are presented as antilogs in tables and figures to facilitate interpretation. Changes in lengths and weights for all individuals, regardless of days of survival, were subjected to one-way analysis of variance (ANOVA). If an ANOVA was significant, a Duncan's multiple range test was used to indicate which means were different. Similar analyses were also conducted using data only from those animals which survived to 16 days. Survivorship, defined as the number of days an individual was observed to be alive, was anlyzed by non-parametric methods. The results of the growth analyses dictated the data groupings used in the statistical tests of survivorship.

RESULTS

Observations

In those beakers containing plant material, shrimp moved to the bottom and began active feeding immediately. Evidence that all foods were fed upon was indicated by full guts in all postlarvae during the entire experimental period, with the exception of the no food treatment. Where substrate was available (i.e. treatments containing *Spartina* epiphytes or detritus) postlarvae were usually found on or burrowed among the plant material. In all other beakers the shrimp were more often observed swimming in the water column.

Growth

At the end of the 16-day experiment changes in lengths and weights among all individuals were significantly different between treatments (One-way ANOVA; $F=21.00$, $d.f.=15,144$, $P<0.001$ for length; $F=26.22$, $d.f.=15,144$, $P<0.001$ for weight). Comparisons by the Duncan's multiple range test ($\alpha=0.05$), indicated that growth increases were greater in treatments containing *Skeletonema* and epiphytes than those containing no food or *Isochrysis* and detritus, alone or in combination. Changes in lengths and weights for those shrimp surviving to 16 days were also significantly different (One-way ANOVA; $F=10.75$, $d.f.=11,86$, $P<0.0001$ for length; $F=10.45$, $d.f.=11,86$, $P<0.0001$ for weight). A posteriori comparisons indicated significant differences between treatments with *Skeletonema*, alone and in combination, and *Spartina* epiphytes in the absence of *Skeletonema* ($\alpha=0.05$, Duncan's multiple range test).

Therefore, the combined results revealed three distinct groups in both length and weight analyses (Table 2). Growth of the shrimp within each group was dependent on a principal food source (i.e., Skeletonema costatum or Spartina epiphytes). The greatest increases in length and weight were attained in Skeletonema costatum treatments (Skeletonema Group) followed by treatments containing Spartina epiphytes without Skeletonema (Epiphyte Group) (Table 3). Essentially no change occurred among shrimp in Isochrysis sp., Spartina detritus, or no food (Isochrysis-Detritus-No Food Group) treatments (Table 3). These results suggested that the presence of certain plant materials did not have additive or synergistic effects on growth.

Growth of postlarvae in the Skeletonema and Epiphyte Groups accelerated after the first four days (Figs. 1 and 2). During the initial period postlarvae in Skeletonema treatments grew at a rate of 0.03 mm/day and 0.2 mg/day (n=74), and individuals in epiphyte treatments increased at a rate of <0.01 mm/day and 0.05 mg/day (n=38). Following this initial interval, growth rates increased to 0.15 mm/day and 0.5 mg/day (n=67) for Skeletonema Group individuals and 0.06 mm/day and 0.2 mg/day (n=31) for Epiphyte Group individuals. Shrimp in the Isochrysis-Detritus-No Food Group exhibited neither significant increases nor decreases in both length and weight during the course of the experiment (Figs. 1 and 2).

The pattern of change-in-weight versus change-in-length was similar between the Skeletonema and Epiphyte Groups, with weight

increasing faster than length. Weight increased an average of 4.4 times faster than length in the Skeleto..ia Group and 5.0 times faster in the Epiphyte Group. In addition to length and weight increases, molting occurred. Exuviae were occasionally found in Skeletonema and epiphyte treatments, but were rapidly consumed thus restricting monitoring of molting frequencies. Exuviae were also present in Isochrysis and Spartina detritus treatments throughout the course of the experiments indicating that growth and ecdysis may not be strictly related. Individuals in the Isochrysis-Detritus-No Food Group frequently had soft exoskeletons.

Survivorship

To test whether survivorship paralleled growth, the mean number of days surviving was compared among the Skeletonema, Epiphyte, and Isochrysis-Detritus-No Food Groups (Fig. 3). All individuals were ranked according to the number of days survived. Significant differences in overall survivorship were found among the three groups (Kruskal-Wallis Test, χ^2=33.24, d.f.=2, n=160, P<0.0001). No significant difference in survivorship, however, was found between the Skeletonema and Epiphyte Groups (Wilcoxon 2-Sample Test, z=-0.5317, P=0.60). Therefore, survivorship was significantly higher in the Skeletonema and Epiphyte Groups as compared to the Isochrysis-Detritus-No Food Group.

Survivorship after 16 days among treatments in the Skeletonema and Epiphyte Groups was 60% to 100% (Table 2) with mean days survived

ranging from 10.6 to 16.0 (Fig. 3). Of mortalities which occurred in the Skeletonema and Epiphyte Groups, many (41%) occurred within the first four days. Deaths during this initial period were probably caused from injuries or stress resulting from netting, transporting, and handling. Survivorship in the Isochrysis-Detritus-No Food Group was 0% to 40% (Table 2) and mean days survived was 7.4 to 13.6 (Fig. 3).

Comparisons of mean number of days surviving among treatments containing only single food sources resulted in highly significant differences (Kruskal-Wallis Test, χ^2=18.99, d.f.=3, P<0.005). However, excluding Isochrysis and testing for differences between Skeletonema, epiphytes, and detritus resulted in non significance (Kruskal-Wallis Test, χ^2=3.09, d.f.=2, P>0.10). This indicated that detritus, even without promoting growth, provided the shrimp with nutrients which extended survivorship beyond the level obtained on Isochrysis.

DISCUSSION

These experiments indicated that in some instances postlarval brown shrimp were able to survive and maintain a positive rate of growth for 16 days on diets consisting exclusively of plant material. That diatoms contribute to the natural diet of postlarval shrimp was supported by the increases in length and weight obtained on Skeletonema costatum. The high survivorship and positive growth rates associated with the Spartina epiphytes diet indicated, as suggested by Condrey et

al. (1972), that this food may be more nutritionally important than previously thought. Spartina detritus, the nutritional value of which has been questioned (Jones, 1973; George, 1978; Weinstein, 1979; Chong & Saskemur, 1981; Moriarty & Barclay, 1981), did not promote growth of postlarvae. Overall these results may have substantial relevance considering the abundances and availability of algae as food in Spartina marshes (Blum, 1968; Lowe & Cox, 1978; Pomeroy et al., 1981), and the evidence that P. aztecus consumes these food sources naturally (Jones, 1973; Kitting et al. 1984).

Although length and weight increases were more rapid in Skeletonema than in epiphytes, the overall growth patterns in the two treatments were the same. Diatoms were present in both treatments lending further support to the hypothesis that diatoms are important in the natural diet of postlarval shrimp. Skeletonema is a chain-forming planktonic diatom while many of the algae that comprise the epiphyte assemblage are branching colonial forms. This hypothesis was further supported by preliminary field caging experiments which showed rapid growth and high survivorship of shrimp in treatments which included diatom rich phytoplankton (Wellington & Gleason, in prep.).

Field studies have reported growth rates of approximately 1.1 mm/day (reviewed by Knudsen et al., 1977) while the maximum growth rate achieved with Skeletonema in this study was 0.12 mm/day. However, since animal material was not included in our experimental diets and growth measurements in field studies have usually involved older

individuals, this was not an appropriate comparison. In addition, laboratory studies using animal diets have found growth of postlarvae to be slow initially, followed by more rapid increases (Zein-Eldin, 1963; Zein-Eldin & Griffith, 1966; Fry & Arnold, 1982). The ability of shrimp to survive and grow on plant diets indicated that these materials can provide a maintenance diet during periods when other foods needed for rapid growth are not available. This strategy, of switching to feed on the dominant plant or animal components, has been found to occur in the planktonic copepod Calanus pacificus (Landry, 1981). If this flexible feeding mode occurs with postlarval brown shrimp, it could provide a distinct advantage in increased survivorship.

It is unlikely that P. aztecus is a strict herbivore because the growth rates measured in the present study were substantially lower then those reported in previous laboratory studies where postlarval shrimp were fed Artemia (Zein-Eldin, 1963; Zein-Eldin & Griffith, 1966; Fry & Arnold, 1982). Plant detritus may still contribute to nutrition indirectly through postlarval feeding on detritivores such as nematodes and polychaetes. This was supported by preliminary experiments which indicated significant reductions in nematode densities when shrimp were present in detritus treatments (see methods). While shrimp did not survive on Spartina detritus, other types of detritus may be nutritionally useful. For example, detritivores such as the polychaete, Capitella capitata, and nematode, Diplolaimella chitwoodi,

assimilate seagrass and algal detritus to a greater degree than Spartina detritus (Findlay, 1982; Findlay & Tenore, 1982).

Molting without growth occurred in detritus and Isochrysis, but was absent in no food treatments. Although molting in crustaceans is generally associated with an increase in body volume (Russell-Hunter, 1979), no increases in either length or weight were detected in these treatments. Apparently the stimuli which triggered ecdysis were unrelated to growth.

Neither additive nor synergistic effects occurred when postlarvae were provided with combinations of foods. This suggests either preferential feeding, differential assimilation, or some association between the two. Accordingly, the data imply the following preference-assimilation hierarchy: Skeletonema costatum > Spartina epiphytes > Spartina detritus = Isochrysis sp. However, this hierarchy may not result from preferences and assimilation alone, but may also involve foraging times and ingestion rates. Experiments, described in Chapter 3, test this hypothesis by monitoring feeding preferences and assessing assimilation rates. Assuming that the foraging times and ingestion rates are the same for all individuals, then differential assimilation is expected.

In some instances postlarval brown shrimp survived and achieved growth on plant sources indicating possible plant contributions in the natural diet. Perhaps algal epiphytes on Spartina, especially the colonial diatoms, as suggested by Condrey et al. (1972), and planktonic

diatoms such as Skeletonema, are important nutritional resources for brown shrimp in marsh ecosystems. Recent field sampling has found Skeletonema to be one of the dominant algal species in Galveston marshes with densities up to 1000 cells/ml not uncommon (S.R. Indelicato, pers. com.). Spartina detritus, and its associated epibenthic blue-green algae, however, appear to provide little or no direct benefits to growth. Preliminary experiments indicated that the resistant gelatinous outer coat in one species of benthic blue-green algae present in the detrital community, Aphanothece stagnina, prevents the postlarvae from successfuly penetrating and feeding on this plant.

TABLE 1

Macrofaunal and meiofaunal abundances in random samples of epiphytic and detrital material before and after being thoroughly washed with filtered (5 μm) seawater. Values represent means with one standard error of the mean in parentheses. One sample equals 1 ml of plant material. The plant materials remaining after processing were used in the feeding experiments.

	N	Nematodes	Oligochaetes & Polychaetes	Copepods
Before Processing:				
Spartina Epiphytes	4	157.5 (16.4)	0.3 (0.3)	9.3 (1.0)
Spartina Detritus	4	78.0 (4.5)	4.0 (1.6)	3.0 (1.1)
After Processing:				
Spartina Epiphytes	4	3.0 (0.8)	0.0 (0.0)	0.3 (0.3)
Spartina Detritus	4	2.3 (0.5)	0.3 (0.3)	0.0 (0.3)

TABLE 2

Growth of postlarval Penaeus aztecus reared 16 days on various plant materials. Values represent means with one standard error of the mean in parentheses. In all treatments initial n=10. Final lengths and weights between the three groups were significantly different by Duncans Multiple Range Test (α=0.05). Skel=Skeletonema costatum, Iso=Isochrysis sp., Epi=Spartina epiphytes, Det=Spartina detritus.

Treatment	Final N	Percent Survival	Mean Length (mm) Initial	Final	Mean Weight (mg) Initial	Final
Skeletonema Group						
Skel, Iso, Epi, Det	10	100	12.42 (.11)	14.55 (.48)	7.8 (.2)	15.9 (.8)
Skel, Epi	8	80	12.43 (.08)	14.64 (.15)	8.0 (.2)	16.0 (.6)
Skel, Epi, Det	6	60	12.54 (.11)	14.63 (.53)	8.2 (.2)	16.4 (1.8)
Skel, Det	7	70	12.34 (.09)	14.29 (.30)	7.8 (.2)	14.6 (1.1)
Skel, Iso	9	90	12.56 (.10)	14.48 (.20)	8.6 (.3)	15.3 (.7)
Skel	9	90	12.58 (.12)	14.44 (.28)	8.5 (.3)	15.3 (1.1)
Skel, Iso, Epi	10	100	12.58 (.17)	14.49 (.19)	8.5 (.3)	15.1 (.7)
Skel, Iso, Det	8	80	12.48 (.14)	14.07 (.15)	8.2 (.3)	13.6 (.5)
Overall Mean	**8.4**	**84**	**12.51 (.04)**	**14.46 (.09)**	**8.2 (.1)**	**15.2 (.8)**
Epiphyte Group						
Epi, Iso	6	60	12.51 (.08)	13.35 (.03)	8.1 (.2)	11.4 (.2)
Epi	8	80	12.83 (.17)	13.56 (.22)	9.0 (.4)	11.8 (.7)
Epi, Iso, Det	10	100	12.59 (.13)	13.14 (.16)	8.3 (.3)	10.8 (.4)
Epi, Det	7	70	12.65 (.15)	13.35 (.24)	8.7 (.4)	11.2 (.7)
Overall Mean	**7.8**	**78**	**12.65 (.07)**	**13.36 (.09)**	**8.5 (.2)**	**11.3 (.3)**
Isochrysis-Detritus-No Food Group						
Iso	0	0	12.22 (.16)	0	7.6 (.4)	0
Det	4	40	12.59 (.16)	12.44 (.08)	8.5 (.3)	7.7 (.5)
Iso, Det	0	0	12.53 (.15)	0	8.4 (.3)	0
No Food	0	0	12.45 (.08)	0	8.2 (.3)	0
Overall Mean	**1.0**	**10**	**12.45 (.07)**	**12.44 (.08)**	**8.2 (.2)**	**7.7 (.5)**

TABLE 3

Changes in lengths and weights and growth rates after 16 days for postlarval Penaeus aztecus reared on plant diets. Values represent means with one standard error of the mean in parentheses. See legend in Table 2 for group designations.

Group	Final N	Change in Length (mm) (final - initial)	Rate mm/day	Change in Weight (mg) (final - initial)	Rate mg/day
Skeletonema	67	1.95 (.07)	0.12 (.01)	7.0 (.3)	0.4 (.02)
Epiphyte	31	0.72 (.08)	0.05 (.01)	2.S (.2)	0.2 (.01)
Isochrysis-Detritus-No Food	4	-0.16 (.06)	--------	-0.8 (.3)	-------

Figure 1: Changes in length (Day x - Initial) for postlarval Penaeus aztecus reared 16 days on plant diets. Consecutive measurements were taken on all surviving individuals at 4-day intervals. See Table II for group designations. Bars denote 95% confidence intervals.

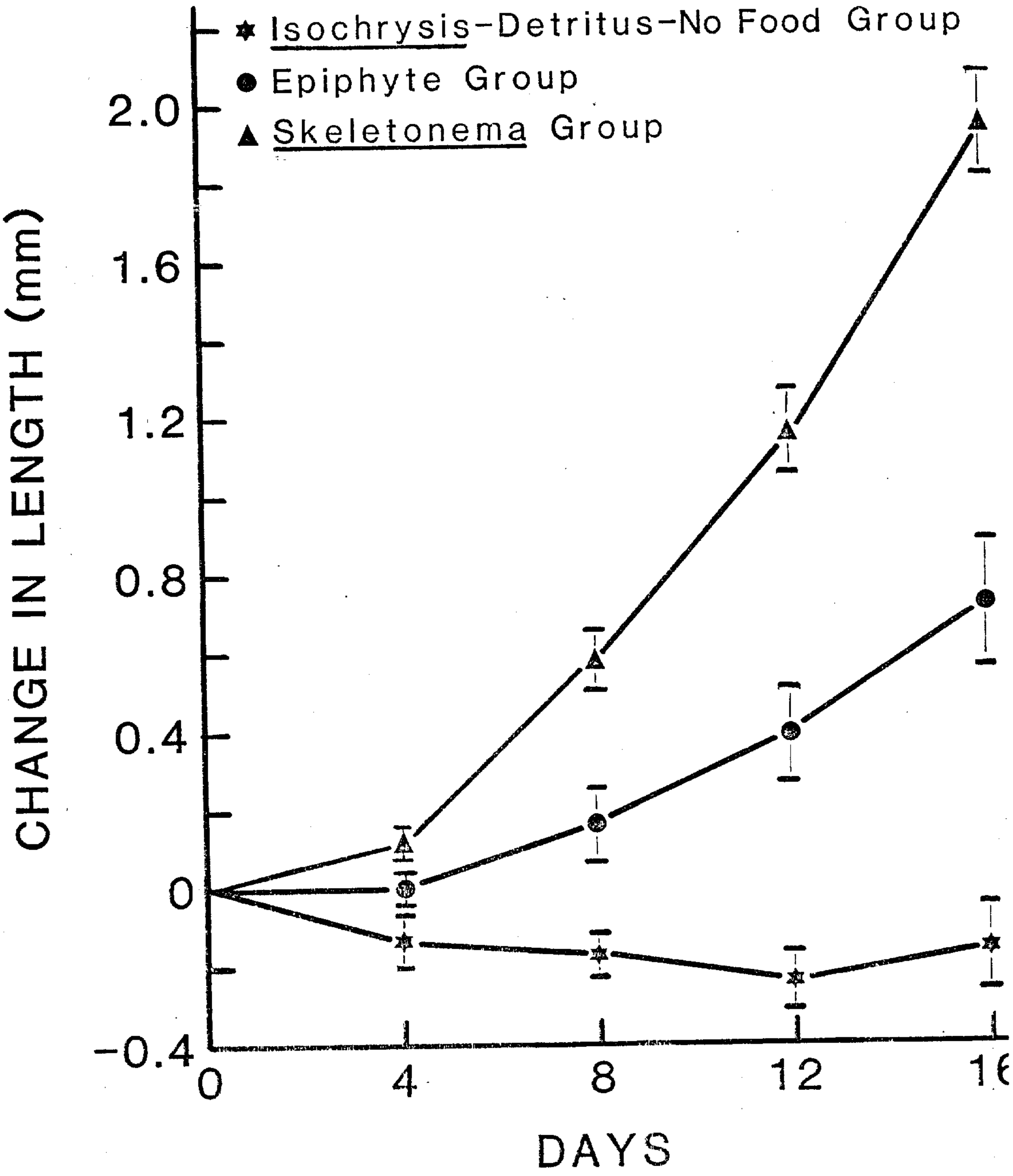

★ Isochrysis-Detritus-No Food Group
● Epiphyte Group
▲ Skeletonema Group
CHANGE IN LENGTH (mm)
2.0
1.6
1.2
0.8
0.4
0
-0.4
0
4
8
12
16
DAYS

Figure 2: Changes in weight (Day x - Initial) for postlarval Penaeus aztecus reared 16 days on plant diets. Consecutive weights were taken on all surviving individuals at 4-day intervals. See Table II for group designations. Bars denote 95% confidence intervals.

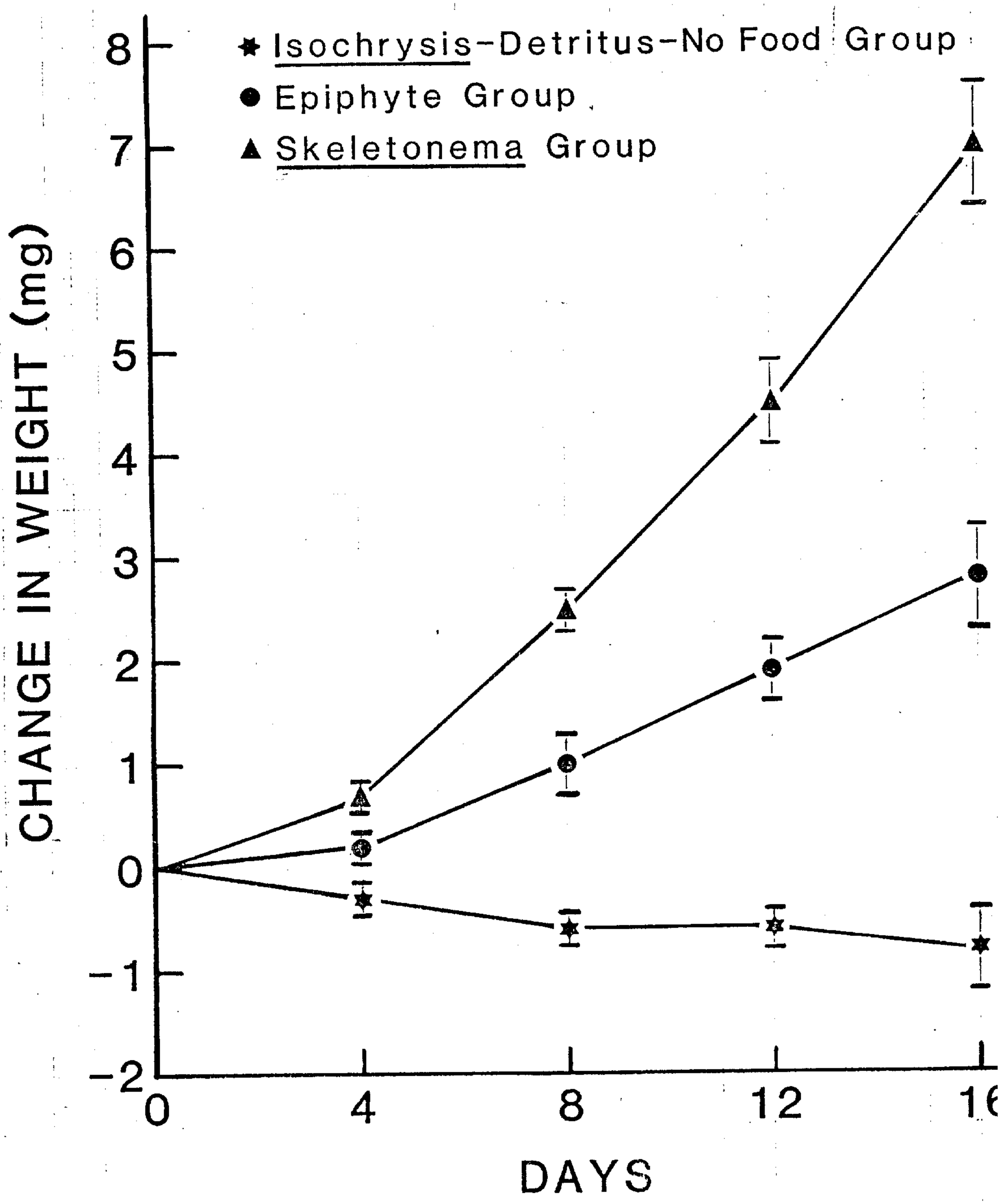
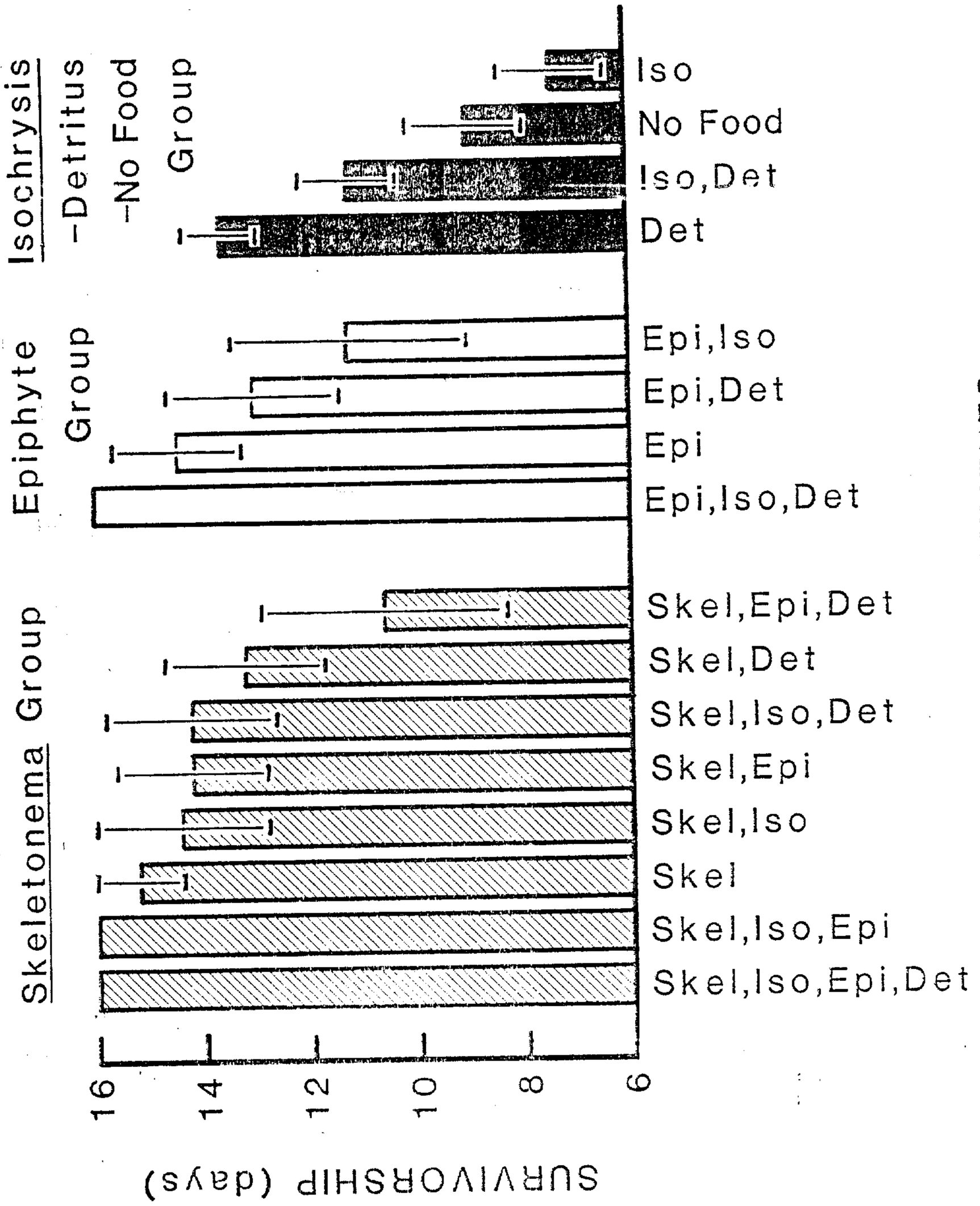

Isochrysis-Detritus-No Food Group
Epiphyte Group
Skeletonema Group
CHANGE IN WEIGHT (mg)
8
7
6
5
4
3
2
1
0
−1
−2
0
4
8
12
16
DAYS

Figure 3: Days of survival for postlarval Penaeus aztecus exposed to all possible combinations of four plant materials. The groupings are based on the results of the growth analyses. Means: Skeletonema Group = 14.2 ± 0.6 s.e. (n=80), Epiphyte Group = 13.7 ± 0.8 s.e. (n=40), Isochrysis-Detritus-No Food Group = 10.3 ± 0.6 s.e. (n=40). Survivorship in the Skeletonema and Epiphyte Groups was significantly higher than in the Isochrysis-Detritus-No Food Group (Kruskal-Wallis Test and Wilcoxon 2-Sample Test). All bars represent means based on 10 individuals. Intervals, where appropriate, represent one standard error of the mean. Skel = Skeletonema costatum, Iso = Isochrysis sp., Epi = Spartina epiphytes, Det = Spartina detritus.


Isochrysis Group
-Detritus
-No Food
Iso
No Food
Iso,Det
Det
Epiphyte Group
Epi,Iso
Epi,Det
Epi
Epi,Iso,Det
Skeletonema Group
Skel,Epi,Det
Skel,Det
Skel,Iso,Det
Skel,Epi
Skel,Iso
Skel
Skel,Iso,Epi
Skel,Iso,Epi,Det
16
14
12
10
8
6
SURVIVORSHIP (days)
TREATMENTS

CHAPTER 3

CARBON ASSIMILATION RATES AND FOOD PREFERENCES

INTRODUCTION

The criterion upon which to evaluate the importance of potential food sources is a complex problem. Optimal foraging theory predicts that to attain an optimal diet, animals will maximize their net energy gain per unit foraging time (Emlen, 1966; MacArthur and Pianka, 1966; Pyke et al., 1977). It is assumed, therefore, that organisms will preferentially consume those foods with the highest caloric content (Emlen, 1966). As suggested by Paine and Vadas (1969), this may not be an accurate prediction of food value since some calories may be present as compounds which are indigestible to the organism. Studies relating food preferences and caloric content have provided evidence both in support of (Stein, 1977) and in opposition to (Paine and Vadas, 1969; Carefoot, 1973; Vadas, 1977; Nicotri, 1980; Jensen, 1983) this general hypothesis. Other factors, including predator avoidance (Stein and Magnuson, 1976; Sih, 1980; Sih, 1982; Edwards, 1983) and maintenance of a nutritionally balanced diet (Belovsky, 1978; Kitting, 1980; and others) may be as important as energy content.

In salt marshes, where plant origins are often obscurred by the detrital base (Haines, 1976;1977; Hackney and Haines, 1980) and direct observations of feeding are rarely possible (Gleason, pers. obs.), evaluations of food resource utilization are confounded further. Recent studies in marine ecosystems have used delta C-13 analysis to investigate feeding and food webs (McConnaughey and McRoy, 1979a; 1979b; Kneib et al., 1980; Stephenson and Lyon, 1982; Hughes and Sherr,

1983; Rau et al., 1983; Fry, 1984). This technique is based on the stable carbon isotope, ^{13}C, which accounts for approximately 1% of the total carbon in the environment. An examination of various photosynthetic organisms indicates that primary producers vary in their ability to assimilate ^{13}C during photosynthesis (Smith and Epstein, 1971; O'Leary, 1981). This variability results in each photosynthetic species being characterized by a specific $^{13}C/^{12}C$ ratio known as delta C-13. Animals feeding directly on plant sources assimilate this carbon, without significantly altering the carbon isotopic composition, resulting in eventual equilibrium in the $^{13}C/^{12}C$ ratio between the animal tissues and the diet carbon (Deniro and Epstein, 1978; Haines and Montague, 1979). If a group of animals with a particular delta C-13 value are fed an assimilable food source of significantly different value, the assimilation rates can be monitored by periodically quantifying the $^{13}C/^{12}C$ ratio in the animals to determine the time necessary for the tissue and diet carbon to equilibrate (Fry and Arnold, 1982). The interval necessary for this change to occur is subject to many variables including the type of tissue analyzed (Tieszen et al., 1983) and the ability of the organism to assimilate the diet carbon (Fry and Arnold, 1982). The advantage of the delta C-13 methodology rests in its ability to provide a natural label, in many instances, allows assimilation to be evaluated directly without previous manipulation of the carbon composition in either the food or the consumer.

The life-cycle of postlarval Penaeus aztecus Ives includes immigration to estuaries from offshore waters (Williams, 1959; Gunter, 1961; Weinstein, 1979). This situation provides a unique opportunity to study the benefits provided by potential food sources, in terms of growth and assimilation, and the relationship between these variables and food preferences. These shrimp must adapt their feeding behavior as they switch from a planktonic existence to one of occupying shallow marsh or seagrass habitats (Fry, 1981). Field studies conducted in these areas have indicated that penaeid shrimp consume plant detritus (Jones, 1973; George, 1978; Chong and Sasekumar, 1981), benthic algae (Jones, 1973; Brisson and Pace, 1978; Hughes and Sherr, 1983), epiphytic algae on plants (Kitting et al., 1984), and planktonic algae (Hughes and Sherr, 1983). However, the large amounts (up to 50%) of unidentifiable debris normally found in gut contents has prevented accurate determinations of the percent composition of these food items in the diet (Jones, 1973; George, 1978; Chong and Sasekumar, 1981). In Spartina alterniflora salt marshes, both laboratory (Giles and Zamora, 1973) and field (Zimmerman et al., 1984) studies have indicated that P. aztecus selects for vegetated areas. In addition, recent experiments investigating herbivory have found that postlarval P. aztecus can survive up to 16 days, and in some instances grow on plant sources from the marsh without augmentation of animal protein (Chapter 2). However, the rates at which these materials are assimilated and their relations to preferences have not been evaluated.

In the present study assimilation patterns, followed with the delta C-13 technique, were compared to changes in weight and feeding preferences for postlarval P. aztecus reared on representative salt marsh plant materials. The purpose was to more fully determine the potential for postlarval P. aztecus to use salt marsh plants and to further define the feeding patterns of this species. Specifically, I attempted to delineate whether preferences for plant foods exist in P. aztecus, and if so, whether these preferences are based solely on growth benefits and assimilation rates.

METHODS AND MATERIALS

Assimilation Experiments

1. Collection and Culture of Shrimp

Four types of representative salt marsh plant materials were used for assimilation studies: Skeletonema costatum (Greville) Cleve (a planktonic diatom), Isochrysis sp. (a flagellated green alga), Spartina alterniflora detritus (decaying vascular plant tissue mixed with the blue-green alga Aphanothece stagnina), and Spartina epiphytes (consisting of many species of blue-green algae, green algae, and diatoms). The procedures for collection and identification of postlarvae, preparation of plant materials, and culturing of animals were as outlined in Chapter 2 with the following modifications: a) assimilation was assessed in only 6 treatments (24 replicates per treatment); four containing a single food source, one with a mixture of

all foods, and one with no food; b) at 4-day intervals up to 16 days, 6 animals were removed, weighed on a microbalance to the nearest 0.2 mg, measured (rostrum-telson) under a dissecting scope to the nearest 0.1 mm, and sacrificed for carbon isotope analysis.

2. Preparation of Tissue Samples

a. Shrimp

Material in the gut of the shrimp represented a nonassimilable or recently ingested carbon contaminant which may have had delta C-13 values distinctly different from that found in the tissues. Therefore, after weighing and measuring, individual postlarvae were placed in beakers containing sea water until their intestinal tracts were voided (normally 3 to 4 hours). Shrimp were killed by freezing and stored at -10° C until further processing. Once thawed, shrimp were treated for 5 minutes with dilute acid (5% phosphoric acid) to remove any remaining carbonates (Haines and Montague, 1979; Fry and Arnold, 1982) and dried at 60° C for a minimum of 48 hours. Dried animals were weighed on a microbalance to the nearest 0.2 mg and stored in a bell jar dessicator containing silica gel until removed for CO_2 gas preparation.

b. Plant Materials

Samples of plant material were taken initially and at 4-day intervals throughout the experiment. Beakers containing plant material, without shrimp, were used to control for possible isotopic changes in the food sources over the 4-day intervals. Spartina detritus and Spartina epiphyte samples were washed free of

contaminating meiofauna by a procedure outlined previously (Chapter 2), while Skeletonema and Isochrysis samples were filtered onto 1.2 µm GF/C grade Whatman glass fiber filters. These filters had been precombusted at 500° C for 2 h to eliminate any foreign organic matter. All plant samples were dried at 60° C for a minimum of 48 h and stored in a dessicator as indicated above.

3. Carbon Isotope Analysis

a) Preparation of CO_2

Combustion tubes 15.2 cm long were cut from 91.5 cm lengths of 9 mm diameter quartz or Vycor tubing and sealed off at one end. Approximately 90-100 mg of CuO and 20 mg of silver wool were added, after which the tube and contents were baked at 550° C for 1 hour to remove organic contaminants (Sofer, 1980). When cool to the touch, one plant or animal sample was added to each tube. Since whole body analysis most accurately depicts the actual delta C-13 value in animals (Deniro and Epstein, 1978), entire shrimp were prepared for combustion. Spartina epiphyte, Spartina detritus, and shrimp samples were loaded directly, while filter pads containing Skeletonema or Isochrysis were wrapped in 2 cm^2 copper foil to prevent fusion of the glass fibers with the quartz or Vycor. Tubes were attached individually to a vacuum line by means of an Ultra-Torr union, evacuated to $<10^{-2}$ mbar and sealed with an oxygen-gas torch. Tissue samples within evacuated tubes were combusted at approximately 850° C for 1 hour. Though past researchers have combusted samples at 550-590° C (Sofer, 1930; Schroeder, 1983;

Fry, 1984; Kitting et al., 1984), 850° C was chosen because of recent reports indicating incomplete combustion at the lower temperature (Boutton et al., 1983).

After cooling to room temperature, sealed tubes were loaded back to back in 0.5 inch o.d. copper tubing closed at one end with a 0.5 inch Ultra-Torr cap. Up to 15 tubes were introduced into 2.3 m lengths of copper pipe. Glass wool, placed between each tube, prevented movement of the tubes and broken glass. After loading, the copper pipe was attached to a vacuum line by means of a 0.5 inch Ultra-Torr union and evacuated to $<10^{-2}$ mbar. Gases were released by crushing the pipe with a pair of pliers in the area of the glass tube. This modified tube cracking technique is faster and easier then previous methods (Desmarais and Hayes, 1976). Once released into the vacuum line, the gas was purified (Fig. 1). Water was removed by condensation in an ethanol-dry ice trap, nitrogen and sulfur oxides were eliminated by passage over copper coils heated to 230° C, CO_2 was collected with liquid nitrogen, and any gases not condensed with nitrogen were pumped away.

b. CO_2 Analysis

Purified CO_2 samples were analyzed with a Micromass 602C mass spectrometer at the Rice University Stable Isotope Facility. All carbon values were calculated according to the equation given by Craig (1953):

$$\text{Delta C-13 in } {}^{o}/_{oo} = \left(\frac{C^{13}/C^{12}\ \text{sample} - C^{13}/C^{12}\ \text{standard}}{C^{13}/C^{12}\ \text{standard}} \right) \cdot 1000$$

where the standard (referred to as PDB) is that ratio found in the calcareous fossil Belemnitella americana of the Pee Dee formation of South Carolina. Since this fossil is enriched in ^{13}C relative to biological tissues, the above formula normally results in negative delta C-13 values. The closer the value is to 0, the more enriched is the sample in ^{13}C. Preliminary analyses indicated that the ratio of $^{13}C/^{12}C$ was relatively homogeneous in ground tissue from the salt marsh plant Spartina alterniflora. Therefore, this material was used as a working standard to locate variability in delta C-13 values due to machine error and CO_2 combustion procedures. At least one Spartina sample was analyzed on each day that the mass spectrometer was used. Replicate analyses of experimental plants were also taken to detect carbon ratio variability within treatments.

Preference Experiments

Feeding preferences were assesed only with those materials which had been previously found to promote growth (i.e., Skeletonema costatum and Spartina epiphytes) (Chapter 2). Postlarval P. aztecus were captured in the surf zone, brought into the laboratory, and acclimated for 4 days in beakers containing a combination of Skeletonema and epiphytes. The exact conditions of culture have been described previously (Chapter 2).

Food preference experiments were conducted in 90 x 15 mm petri dishes. Skeletonema and epiphytic plant materials were first suspended in sea water and then filtered onto 35 mm diameter 1.2 µm Whatman GF/C

grade glass fiber filters. Each dish contained 3 pads (epiphytes alone, Skeletonema alone, epiphytes and Skeletonema together), presented to the shrimp in one of three possible orientations (Fig. 2). A 2.5 cm length of 0.5 inch PVC pipe, placed in the center of each vessel, served as a holding cylinder. Postlarvae were starved for 24 h to 36 h prior to initiation of the experiments. Shrimp were added to the cylinders in groups of 7. Previous observations indicated that displacement from filter pads was not a factor if 7 animals or less were used. Shrimp were acclimated in the holding cylinders for approximately 5 minutes and then released by raising the cylinder. Observations noting the position of each individual were recorded after 10 minutes and then every 5 minutes for a total of 30 minutes (5 observations per replicate). Preliminary experiments showed that all shrimp guts were full after 30 minutes. Preferences were monitored in 10 replicates during the day and 10 at night, for a total of 140 shrimp. The salinity and temperature were maintained at constant levels of 25 $\pm 1^{\circ}$ C and 25 $\pm 1^{\circ}/oo$ during all observations. Day replicates were conducted between 1400 and 1900 hours under Sylvania 40 watt daylight bulbs. Night observations were made between 2100 and 0200 hours with 60 watt Sylvania red bulbs to simulate darkness.

RESULTS

Growth

Growth patterns for the shrimp, displayed in Table 1 and Fig. 3, were similar to those found previously (Chapter 2). Changes in weight (final weight - initial weight) were significantly different among treatments in those animals surviving to 16 days (One-way ANOVA, $F=49.13$, d.f.=3,33, $P<0.001$). Analysis by Duncan's multiple range test ($\alpha=0.05$) indicated three distinct groups. Maximum increases occurred in Skeletonema and combination (all four materials together) treatments followed by beakers containing Spartina epiphytes. Significant increases in weight did not occur in animals fed Spartina detritus. Although no shrimp remained alive for the entire 16 days in either the Isochrysis or no food treatments, increases in weight were not recorded during the 12 days that animals were present (Fig. 3).

Assimilation

Variability in delta C-13 due to machine error and the CO_2 combustion procedure was found to be well within the generally excepted limits. Analyses of ground Spartina tissue resulted in a standard error of only ± 0.02 °/oo (mean delta C-13 = -12.34, n=19). The absolute mean difference in delta C-13 values for replicate samples of plant materials within experimental treatments was found to be 0.10 ± 0.03 °/oo (S.E., n=11). Combining the variabilities due to the entire procedure (i.e., machine error, CO_2 combustion, and plant tissue heterogeneity), resulted in a total standard error of $\pm$ 0.05 °/oo.

The delta C-13 values of the plant materials remained fairly consistent during the experiment. The $^{13}C/^{12}C$ ratios of the plant materials, measured at 4-day intervals, were not significantly different within treatments (One-way ANOVA; F=3.01, d.f.=4,10, P>0.05 for Isochrysis; F=2.56, d.f.=4,19, P>0.05 for detritus; F=0.80, d.f.=4,19, P>0.10 for epiphytes; F=0.79, d.f.=2,12, P>0.10 for Skeletonema). Isochrysis sp. was the most depleted in ^{13}C while Spartina detritus was the most enriched (Table 2). Skeletonema costatum and Spartina epiphytes both had values midway between Isochrysis and detritus and could not be distinguished from each other on the basis of carbon isotopic values alone (Table 2).

Assimilation corresponded to growth in those treatments where the greatest increases in weight occurred (Table 3 and Fig. 4). Comparisons, by the Mann-Whitney U-test, between initial and final shrimp delta C-13 values indicated that significant changes occurred only in beakers containing Skeletonema (U_s=40, n_1=10, n_2=4, P<<0.01) or all plant materials (U_s=40, n_1=10, n_2=4, P<<0.01). Changes in all other treatments were nonsignificant (U_s=31.5, n_1=10, n_2=4, P>0.05 for epiphytes; U_s=30.5, n_1=10, n_2=4, P>0.10 for detritus; U_s=21.5, n_1=10, n_2=4, P>0.10 for Isochrysis; U_s=28.5, n_1=10, n_2=4, P>0.10 for no food). Postlarvae fed Skeletonema alone became approximately 1°/oo enriched relative to their food source. This ^{13}C enrichment commmonly occurs when food sources are assimilated and is the result of ^{12}C being preferentially released during respiration (DeniRo and Epstein, 1978).

All other individuals reared on single plant sources, except those in Isochrysis, remained depleted relative to the respective plant material during the entire experiment.

Assimilation could not be evaluated in Isochrysis treatments. Shrimp in Isochrysis were approximately 1°/oo enriched relative to this algae initially, and throughout the entire 12 days that animals survived. Since growth and survival on Isochrysis followed closely that obtained in no food treatments, assimilation was assumed to be negligible.

Comparisons of the delta C-13 values, at each of the four sampling days, between shrimp in Skeletonema and mixed treatments showed no significant differences (One-way ANOVA, $P>>0.05$ in all cases).

Assimilation patterns represented as percent increase in wet weight as a function of changes in delta C-13 (Fig. 5), followed a power function of the form $y=a+bx^c$, where c is the metabolic constant (Fry and Arnold, 1982). Values for a,b, and c were determined according to the procedures of Fry and Arnold (1982). Briefly, c was calculated by linear regression (Sokal and Rohlf, 1969), using transformed variables, (x,y) to (x^c,y). The initial regression was conducted at c equal to -1 and was followed by subsequent regressions at decreasing increments of 0.01 units for c until the least residual sum of squares was found. At this point a and b were also at their best fit values. Power functions, based on percent increases in wet weight, resulted in nearly identical delta C-13 assimilation curves for shrimp reared in

Skeletonema and combination treatments. Carbon from the diet appeared to have been assimilated at approximately the same rate in both treatments (Fig. 5). The metabolic constants (c), displayed in Table 4, were less than -1 confirming that turnover took place (Fry and Arnold, 1982). Confidence limits (95%) for c were calculated according to Silvert [1979; in Fry and Arnold (1982)]. The large overlap in these confidence limits indicated that the metabolic constants were not different. Comparing the c values, through the use of a t-test, confirmed this conclusion ($P>0.05$). Assimilation based on $T(w)=w^{c+1}$; where $T(w)$ = turnover function, w = percent increase in wet weight, and c = metabolic constant (Fry and Arnold, 1982); was initially rapid, but decreased as the half-life of carbon was approached. In both treatments, the half-life of carbon was reached in the shrimp tissues before the first doubling of weight (Table 4).

Food Preferences

Shrimp were observed to follow the same behavioral patterns regardless of the time of day. Once released from the PVC holding chamber, the shrimp required approximately 5 minutes to acclimate to the petri dish. Turning the red light off and on several times during night trials resulted in no visible affects on the behavior of the animals, whereas switching to daylight bulbs startled the shrimp and invoked rapid swimming. On the basis of these observations, and previous studies indicating the inability of crustaceans to readily perceive red wavelengths (reviewed by Waterman, 1961), it was concluded

that the red light was probably not detected by the postlarval shrimp and was adequate for simulating night conditions.

The number of individuals observed on each substrate is given in Table 5. Preferences for food or substrate did not differ significantly in time between the 5 minute intervals in either the day or night trials (χ^2=16.67, d.f.=12, P>0.10 for day; χ^2=10.28, d.f.=12, P>0.50 for night). Furthermore, pooling the values over the 30 minutes resulted in no significant differences in preference between day and night trials (Table 6). However, preferences for the various foods were found to be significant (Table 6). Although preferences for pads containing Skeletonema and epiphytes together or epiphytes alone were not different, selection for both of these treatments was greater than for Skeletonema alone (Gabriel's Simultaneous Test Procedure, α =0.05). There was no interaction between time of day and treatment (Table 6). The combined results indicated that the postlarvae were not selecting for substrates strictly on the basis of their ability to promote growth or be assimilated.

DISCUSSION

This study demonstrated that plant food sources which promoted growth and were more easily assimilated were not necessarily the preferred items in the diet of postlarval P. aztecus. Growth in P. aztecus was similar to that found previously (Chapter 2), with the greatest changes in weight occurring on a diet of Skeletonema alone or

a combination of all experimental plants. As expected, the plant materials were differentially assimilated, with the most rapid carbon turnover in the tissues of shrimp fed Skeletonema alone or a mixture of all foods. The relationship between weight increases and assimilation rates in these treatments demonstrated the utility of delta C-13 analysis for determining the benefits, to growth, of potential food sources. The ability of P. aztecus to readily assimilate Skeletonema supported the hypothesis that diatoms represent an important food source for developing shrimp (Chapter 2). Feeding preferences did not correlate well with either growth or assimilation indicating that the postlarvae may have a primary preference for substrate which is not directly coupled with plant food value. This behavior may be related to factors involved in switching from a planktonic to a benthic existence.

In a previous assimilation study, conducted by Fry and Arnold (1982), where brown shrimp were fed animal diets, it was suggested that turnover in postlarval P. aztecus was more a function of increases in weight than metabolic maintenance. Although the growth rates in the present experiments, for shrimp fed Skeletonema or a combination of all foods, were generally lower than in this previous study, the metabolic constants and half-lives of carbon were similar in the two investigations. These comparisons indicated that plants may be more important than animal constituents for metabolic maintenance and suggested that postlarvae may need both components to completely

fulfill their dietary requirements. Carbon turnover in the present study occurred in treatments where the greatest increases in shrimp body weight were recorded (i.e., Skeletonema and all foods in combination). This supported the hypothesis introduced by Fry and Arnold (1982), that turnover rates in postlarval P. aztecus are positively correlated with growth rates.

Although shrimp reared on epiphytes of Spartina showed growth, the delta C-13 values for these individuals did not change significantly over the 16 day experiment. This result was explained in several ways. First, casual observations of postlarval feeding behavior indicated that food selection took place at the mouthparts. Food preferences occurring at this stage were not detectable by the design of the preference experiments. Therefore, since epiphytes of Spartina represented a mixture of many species of diatoms, blue-green algae, and green algae; it was possible that the delta C-13 values were the result of the shrimp preferentially feeding on single species or combinations which were more depleted in ^{13}C relative to the entire algal complex. Another possible explanation was that all of the algae of the epiphyte complex were consumed by P. aztecus, but all of them were not assimilable. Thus, the results obtained in this study were possible if the shrimp were only capable of assimilating those algal components with a net delta C-13 value more negative than the overall epiphyte value. Finally, if the algae constituting the epiphyte assemblage were not strongly connected with maintenance metabolism, it was possible

that the increases in weight obtained over the 16 days were not great enough to result in an observable affect on the $^{13}C/^{12}C$ ratio.

There was no evidence of preferential feeding on Skeletonema making it difficult to understand why the assimilation curves were nearly identical for shrimp fed Skeletonema alone or all foods in combination. It was possible that in the combinations treatments the postlarvae were feeding on an assortment of foods which resulted in delta C-13 values resembling Skeletonema alone. Although individually certain food sources were not assimilated, their incorporation into the body tissues may have been enhanced when consumed in conjunction with other plants. Many combinations could have mimicked Skeletonema alone. For example, consumption of 50% Isochrysis and 50% detritus would have resulted in a delta C-13 value of -17.5 °/oo, given that both materials were assimilated equally. However, because previous growth experiments indicated no additive or synergistic effects for shrimp reared on combinations of these plants (Chapter 2), this was an unlikely conclusion. A more plausible explanation was that the postlarvae possessed an assimilation threshold, a point at which further consumption of a usable food source resulted in its passage through the gut as waste. In treatments containing all foods, if the shrimp preferentially settled on epiphytes of Spartina and began feeding, inadvertently Skeletonema would have been consumed. A threshold model predicts that enough Skeletonema was consumed to at least meet the minimum assimilation requirement. Based on this conclusion, if the

condition within the salt marsh is such that the rate at which the shrimp encounter quality foods is rarely predictable, then it is likely that P. aztecus has compensated by developing a high assimilation efficiency and low assimilation threshold for nutritionally valuable resources. Future experiments should test this threshold model and its relation to postlarval feeding behavior.

The use of delta C-13 analysis to follow the flow of carbon from producer to consumer provides an added dimension to the study of foraging and food resource utilization. This technique, unlike analysis of gut contents, provides a more accurate indication of the diet over the long term and a direct measure of the ability of the animal to utilize materials consumed. In cases where ingestion and excretion are difficult to monitor, delta C-13 analysis represents an alternate method of measuring food assimilability. This technique is especially useful in controlled laboratory and field situations where the possible diet combinations can be readily identified. However, caution must be advised when evaluating carbon isotope data. It is generally accepted that ^{12}C is preferentially released during respiration resulting in a ^{13}C enrichment in animal tissue of approximately 1‰ compared to the diet (Haines and Montague, 1979; McConnaughey and McRoy, 1979a; 1979b; Petelle et al., 1979; Boutton et al., 1983; Rau et al., 1983). The final values obtained for shrimp reared on Skeletonema provided direct evidence that this isotope fractionation occurred in the present experiments. Although this ^{13}C enrichment did not hinder the

interpretation of the data in this study, it could result in improper evaluations in laboratory or field experiments where all of the food sources have very similar delta C-13 values.

The growth and assimilation rates displayed by the shrimp were not significantly different among treatments containing Skeletonema alone or Skeletonema in combination with any of the other plant sources. This result was expected if the shrimp were preferentially feeding on Skeletonema in treatments containing this diatom in association with one or more of the other plant sources. Preferences, however, did not correspond with either growth or assimilation indicating that food selection may not be based solely on apparent nutritional aspects. This result is in agreement with past studies which found no relationship when comparing caloric content (Paine and Vadas, 1969; Carefoot, 1973; Vadas, 1977; Nicotri, 1980; Jensen, 1983) and growth (Nicotri, 1980) to preferences. Due to its darker color and more three dimensional surface, substrates containing epiphytes of Spartina may be more attractive as a refuge from predators than those consisting only of Skeletonema. Additionally, epiphytes of Spartina harbor meiofaunal components (Capter 2) which may be important in the diet of P. aztecus (R.J. Zimmerman, pers. com.). Therefore, these factors, along with palatability, handling times, and micronutrient requirements, may have a higher priority for the survivorship of the postlarvae than preference for nutritionally valuable plant foods.

Predation by fishes is an important source of mortality for P.

aztecus inhabiting Spartina salt marshes (reviewed by Minello and Zimmerman, 1983). This may be especially true for postlarval shrimp which enter the marsh at only 10-12 mm (rostrum-telson). Selection for substrate which provides cover may initially be more important to the survival of these animals than nutritional considerations. Studies on crayfish indicate gravitation toward that substrate which affords the most protection when a predator is present (Stein and Magnuson, 1976; Stein, 1977). Changes in foraging patterns, such as switching to feed in areas of lower food quality or reducing food consumption may also occur in the presence of predators (Stein and Magnuson, 1976; Sih, 1982; Edwards, 1983). For penaeid shrimp inhabiting salt marshes, where many predators rely on visual cues (Minello and Zimmerman, 1983), immediate preference for substrates or complex structures which provide protection, such as Spartina stems, may be a selected behavior for increased survivorship. Therefore, active foraging begins only after suitable cover is located. This conclusion is supported by Giles and Zamora (1973) who noted that juvenile P. aztecus exhibits a strong preference for vegetation even in the absence of predators.

Spartina stems not only decrease the efficiency of certain fish predators, such as pinfish (Logodon rhomboides) and Atlantic croaker (Micropogonias undulatus), on P. aztecus (Minello and Zimmerman, 1983), but epiphytes on these stems also contain high densities of animals which may be important in shrimp nutrition (Chapter 2). Recent experiments indicated that postlarval P. aztecus readily consumes

meiofauna associated with epiphytes (Chapter 2; R.J. Zimmerman, pers. comm.). Therefore, the results of the present study may have actually represented preferences which provide the greatest access to important plant and animal dietary sources in the natural habitat. Future experiments, which include both plant and animal components, investigating changes in foraging patterns and other behavior in response to various levels of predation intensity are needed to clarify the relationship between substrate preferences and food preferences.

TABLE 1

Growth after 16 days of postlarval Penaeus aztecus reared on plant diets. No individuals in the Isochrysis or no food treatments survived the full term. Values represent means with one standard error of the mean in parentheses. Groups with the same letter were not significantly different (Duncan's multiple range test). The combination treatment included all food types.

Treatment	Number of Individuals	Change in Weight (mg)	Growth Rate mg/day	Significance (α=0.05)
Combination	10	6.1 (0.8)	0.4 (0.05)	A
Skeletonema	8	4.8 (0.3)	0.3 (0.02)	A
Epiphytes	7	1.7 (0.2)	0.1 (0.02)	B
Detritus	12	-0.7 (0.2)	0.0	C

TABLE 2

Delta C-13 values for plant materials fed to shrimp. Plant samples were taken initially and every 4 days during the course of the experiment. The values were not significantly different within plant materials over the 16 days (One-way ANOVA, $P>0.05$ for all materials). Values represent means of independent samples with one standard error of the mean in parentheses. Groups with the same letter were not significantly different (Duncan's multiple range test).

Plant Material	N	Delta C-13 Value (°/oo)	Significance ($\alpha=0.05$)
Spartina detritus	24	-15.0 (0.1)	A
Spartina epiphytes	24	-18.3 (0.2)	B
Skeletonema costatum	17	-18.3 (0.2)	B
Isochrysis sp.	15	-20.0 (0.2)	C

TABLE 3

Patterns of $^{13}C/^{12}C$ ratios in postlarval Penaeus aztecus reared on plant diets. Delta C-13 results are compared with the initial shrimp value and the appropriate plant value. All delta C-13 values represent means with one standard error of the mean in parentheses. Sample size for initial values is based on 10 animals, in all other cases N=4 animals/treatment/sampling period. Statistical analyses tested for differences between initial and final delta C-13 values (Mann-Whitney U-test). See Figure 5 for assimilation rates as a function of change in body weight. N.S.=denotes nonsignificant differences (P>0.05).

Treatment	Day	Delta C-13 (°/oo)	Significance	Present ^{13}C -Initial ^{13}C	^{13}C animal -^{13}C food
Initial	0	-19.2 (0.1)	-------	-----	-----
Combination	4	-18.9 (0.3)		0.3	-----
	8	-18.1 (0.3)		1.1	-----
	12	-17.5 (0.2)		1.7	-----
	16	-17.1 (0.1)	P<<0.01	2.1	-----
Skeletonema	4	-18.6 (0.6)		0.6	-0.3
	8	-18.1 (0.2)		1.1	+0.2
	12	-17.5 (0.2)		1.7	+0.8
	16	-17.2 (0.2)	P<<0.01	2.1	+1.1
Epiphytes	4	-19.1 (0.2)		0.1	-0.8
	8	-19.4 (0.1)		-0.2	-1.1
	12	-18.8 (0.2)		0.4	-0.5
	16	-18.8 (0.3)	N.S.	0.4	-0.5
Detritus	4	-18.6 (0.6)		0.6	-3.6
	8	-19.3 (0.4)		-0.1	-4.3
	12	-19.1 (0.1)		0.1	-4.1
	16	-18.9 (0.3)	N.S.	0.3	-3.9
Isochrysis	4	-19.3 (0.1)		-0.1	+0.7
	8	-18.9 (0.2)		0.3	+1.1
	12	-19.1 (0.2)	N.S.	0.1	+0.9
	16	-------		---	----
No Food	4	-19.2 (0.2)		0.0	----
	8	-19.4 (0.1)		-0.2	----
	12	-19.4 (0.2)	N.S.	-0.2	----
	16	-------		---	----

TABLE 4

Metabolic constants and half-lives of carbon for shrimp in treatments where asssimilation occurred. The more negative the metabolic constant, the more rapid the rate of assimilation. The metabolic constants were not significantly different (t-test, $P>0.05$). Initial weight = 100%

Treatment	Metabolic Constant (c)	95% C.L. for c	% Initial Weight at Half-life of Carbon
Combination	-2.29	-1.55, -3.27	171
Skeletonema	-2.63	-2.11, -3.28	153

TABLE 5

Number of animals found on each food source at 5 observation periods. Values at each time interval represent means of 10 independent replicates (7 animals/replicate), with one standard error of the mean in parentheses. Preferences did not vary with time over the 30 minute period during day or night trials (G-test of goodness-of-fit: P>0.10 for day, P>0.50 for night).

Time Elapsed (minutes)	Treatments: Epiphytes Alone	Skeletonema + Epiphytes	Skeletonema Alone	Off Food Substrates
		DAY TRIALS		
10	2.3 (0.4)	2.5 (0.4)	1.2 (0.4)	1.0 (0.3)
15	2.4 (0.3)	2.5 (0.4)	1.0 (0.3)	1.1 (0.2)
20	2.7 (0.4)	2.6 (0.2)	0.7 (0.2)	1.0 (0.3)
25	2.9 (0.5)	2.0 (0.5)	0.4 (0.2)	1.7 (0.3)
30	2.8 (0.4)	2.3 (0.3)	0.2 (0.1)	1.7 (0.2)
		NIGHT TRIALS		
10	2.6 (0.4)	2.1 (0.3)	1.0 (0.3)	1.3 (0.4)
15	3.1 (0.5)	2.8 (0.4)	0.5 (0.2)	0.6 (0.3)
20	2.6 (0.4)	2.8 (0.5)	0.6 (0.2)	1.0 (0.3)
25	3.2 (0.4)	2.4 (0.3)	0.4 (0.2)	1.0 (0.4)
30	3.2 (0.3)	2.5 (0.3)	0.3 (0.2)	1.0 (0.3)

TABLE 6

A. Number of animals found on each food source during day and night observations. Values represent means of 10 independent replicates (7 animals/replicate), observed 5 times. The 5 observations were summed resulting in one data point per replicate for each treatment. A posteriori comparisons by Gabriel's sum of squares simultaneous test procedure indicated that selection for epiphytes alone and Skeletonema and epiphytes together was significantly greater than for Skeletonema alone or off food substrates (α=0.05).

Time	Number of Replicates	Treatments			
		Epiphytes Alone	Skeletonema + Epiphytes	Skeletonema Alone	Off Food Substrates
Day	10	13.1 (1.2)	11.9 (1.2)	3.5 (1.2)	6.5 (0.5)
Night	10	14.7 (1.1)	12.6 (0.9)	2.8 (0.5)	4.9 (0.9)
Overall	**20**	**13.9 (0.8)**	**12.3 (0.7)**	**3.2 (0.6)**	5.7 (0.5)

B. Two-way ANOVA. Time of preference experiments versus substrate type. N.S.=denotes nonsignificant differences (P>0.05).

	d.f.	SS	MS	F-value	Significance
Substrate	3	1588.7	529.57	57.07	P<<0.01
Time	1	0.09	0.09	0.01	N.S.
Substrate x Time	3	30.5	10.17	1.10	N.S.
Error	72	667.8	9.28		

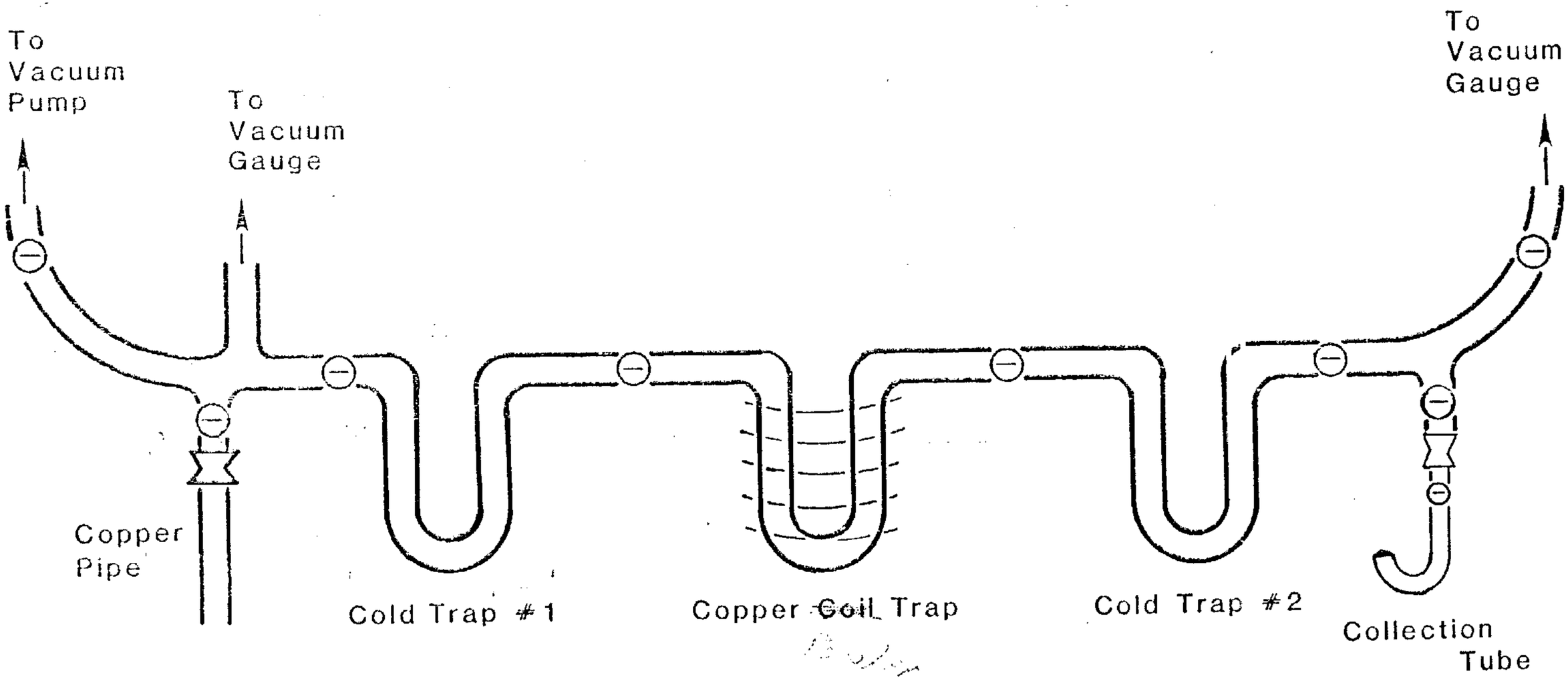
Figure 1: CO_2 gas purification line. Prior to being admitted to the mass spectrometer, CO_2 gas was released into the vacuum line and purified by the following process:

1) Gas was collected in the first cold trap with liquid nitrogen. After 2 minutes the non-condensible gases were pumped away and the liquid nitrogen was replaced by an isopropynol-dry ice slush.

2) While water remained condensed in the first cold trap, the carbon dioxide gas was allowed to pass for 1.5 minutes into the loop containing copper coils heated to 230° C. This section of the line reduced nitrogen and sulfur oxides.

3) The carbon dioxide was collected in the final cold trap with liquid nitrogen. After 2 minutes, non-condensible gases were again pumped away and the nitrogen was replaced by the isopropynol-dry ice slush.

4) Using liquid nitrogen, the purified carbon dioxide was condensed into the collection tube for 2 minutes. After 2 minutes this tube was sealed with a stopcock in order that isotope measurements could be made at a later time. All gases were extracted and analyzed within the same 24 h period.



⊖ Teflon Stopcock

Figure 2: Orientation of filter pads in petri dishes during shrimp feeding preference experiments. Each dish represented one replicate. Out of 10 replicates each arrangement (A,B, and C), was used 3 times with the final orientation randomly chosen. Skel = Skeletonema costatum, Epi = Spartina epiphytes.

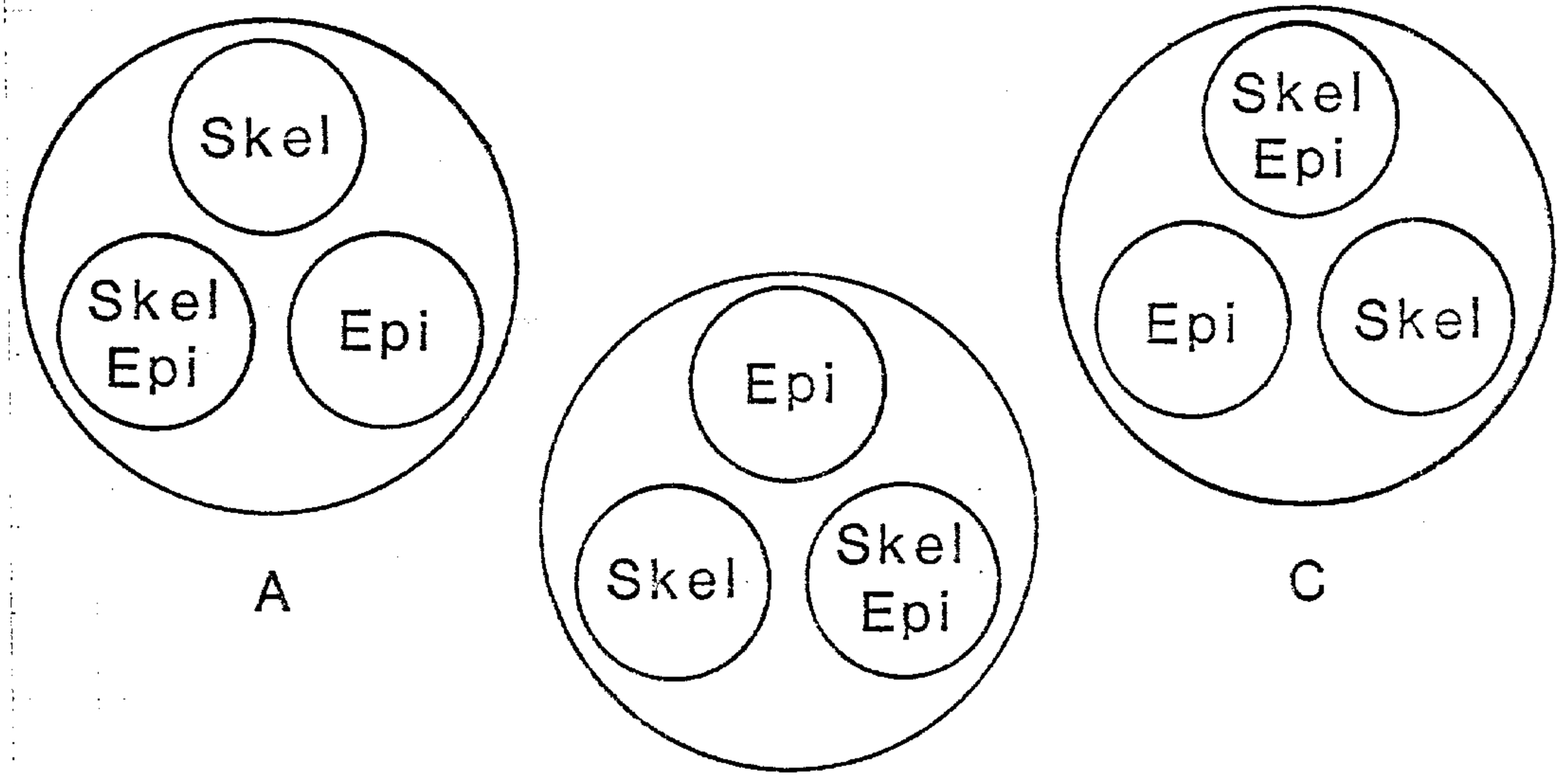

Skel
Skel
Epi
Epi
A
Epi
Skel
Skel
Epi
B
Skel
Epi
Epi
Skel
C

Figure 3: Changes in weight (day x - initial) for postlarval Penaeus aztecus reared 16 days on plant diets. In all cases, except at 16 days, values represent means of 6 individuals. At 16 days n=10 for combination, 8 for Skeletonema, 7 for epiphytes, and 12 for detritus. Vertical bars denote one standard error of the mean.

△ Combination
● Skeletonema
★ Epiphytes
□ Detritus
■ Isochrysis
○ No Food

CHANGE IN WEIGHT (mg)

7
6
5
4
3
2
1
0
−1
−2

0
4
8
12
16

DAYS ELAPSED

Figure 4: Changes in delta C-13 as a function of time for postlarval Penaeus aztecus fed plant diets. All data points, except in the initial sample (n=10), represent means of 4 animals. Vertical bars around the initial value represent one standard error of the mean. For standard errors of all other values refer to Table 3.

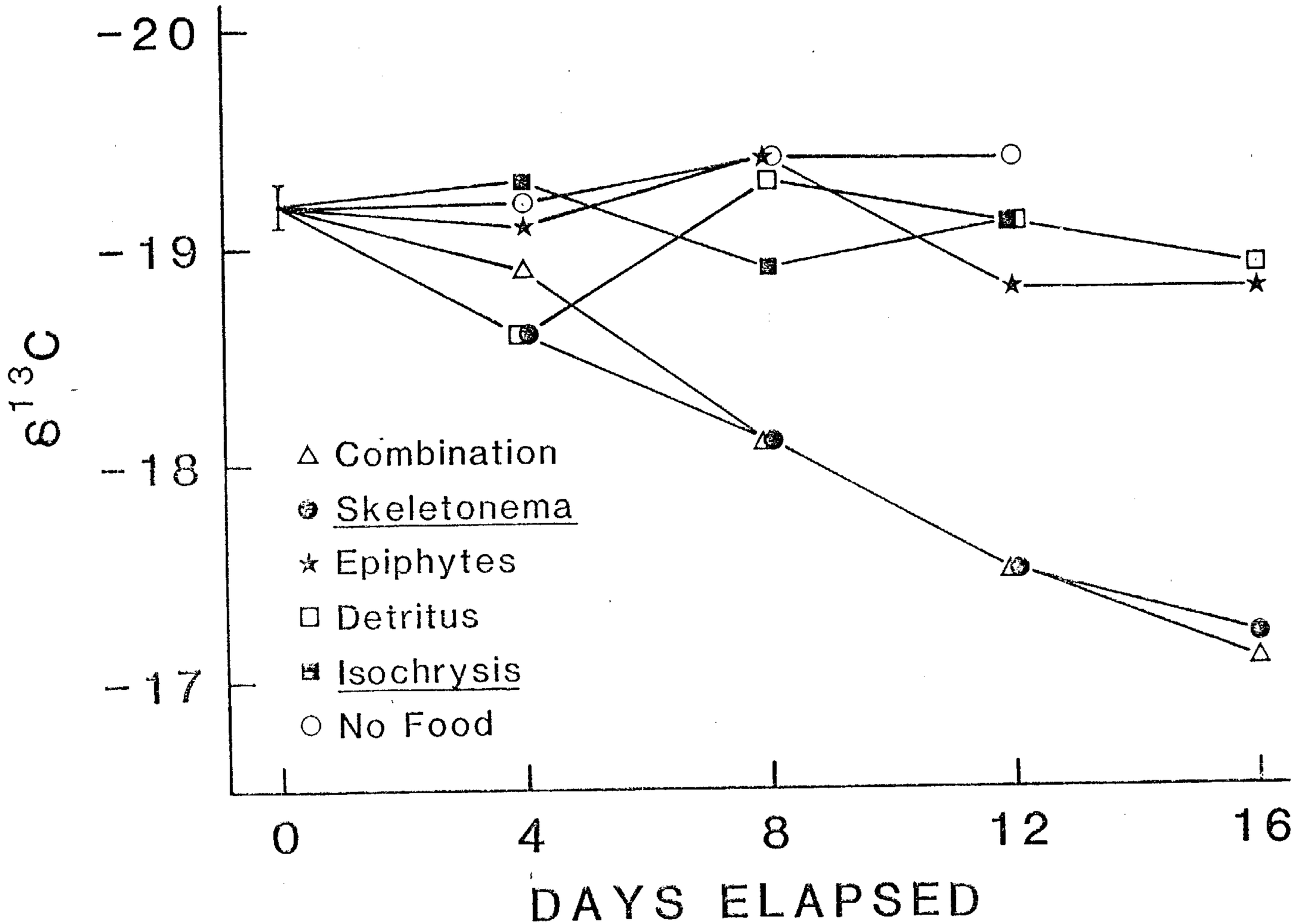
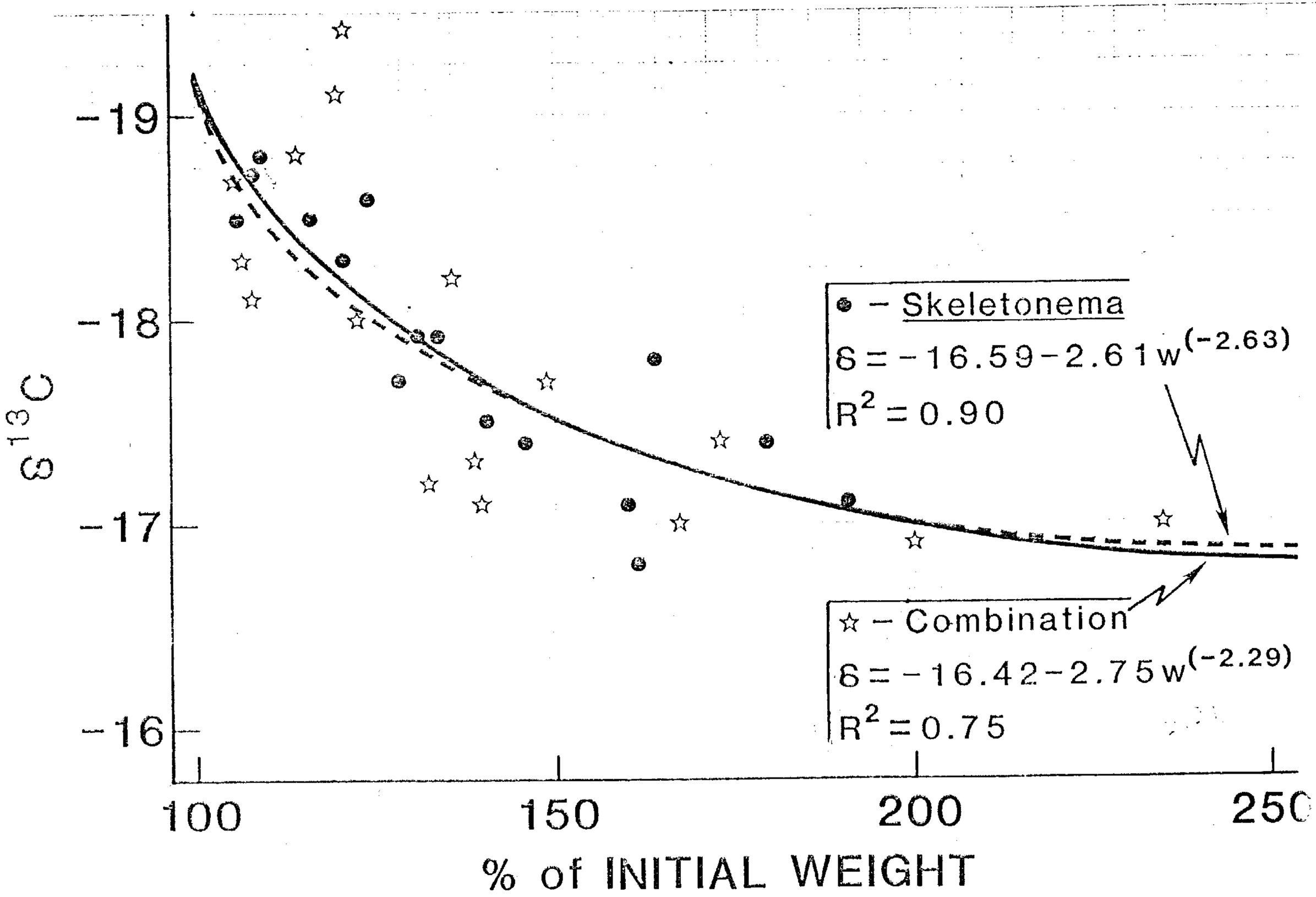

−20
−19
−18
−17
$\delta^{13}C$
△ Combination
● Skeletonema
★ Epiphytes
□ Detritus
■ Isochrysis
○ No Food
0
4
8
12
16
DAYS ELAPSED

Figure 5: Changes in delta C-13 as a function of weight for postlarval Penaeus aztecus fed plant diets. Power curves of the form $y=a+bx^c$, where c=metabolic constant, were fitted to the data (see text for details). The more negative the value of c the more rapid the carbon turnover in the tissues. The metabolic constants were not significantly different (t-test, $P>0.05$).


-19
-18
-17
-16
δ13C
100
150
200
250
% of INITIAL WEIGHT
● - Skeletonema
δ = -16.59-2.61w(-2.63)
R2 = 0.90
☆ - Combination
δ = -16.42-2.75w(-2.29)
R2 = 0.75

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